Application of EnPresso Y Defined for small-scale bioprocess optimization

Application Note

Summary of published results [1-3], by Dr. Antje Neubauer

Introduction

Yeasts such as *Saccharomyces cerevisiae and Komagataella phaffii* (also known as *Pichia pastoris*) have gained significant scientific attention for several industrial bioprocesses. Approaches to accelerate the timeline of process development utilizes high-throughput technology platforms such as microtiter plate cultures, small-scale bioreactors and in parallel fermentation systems. A successful bioprocess application on an industrial scale requires extensive and accurate process control during the process development and a seamless scale-up process. Model-based process control methods and industry-relevant process conditions, such as the implementation of fed-batch strategies at the beginning of the development, are becoming increasingly important.

In this application note, we present four studies demonstrating how EnPresso Y Defined can serve as an effective tool for optimizing yeast processes. EnPresso Y Defined is a pre-sterilized growth system designed to increase the yield of functional proteins expressed in *Komagataella phaffii*. However, the medium composition of the tablets and the controlled growth rate by adding an glucose releasing enzyme is applicable for other yeasts as well.

Methods

[1]: a) a 96-well microplate with F-bottom format (Greiner bio-one, GmbH, Germany) with sandwich covers (Enzyscreen B.V., Haarlem, the Netherlands) for screening of single mutants
b) 12mL cultivation volume in bioREACTOR 48 (2mag AG) was combined with a Tecan Feedom Evo platform

[2] cultivation in 24-deep-well plate (DWP)
[3] a 96-well sensor plate (Presens Precision Sensing GmbH) with sandwich covers (Enzyscreen B.V., Haarlem, the Netherlands)

Strains:

[1]: *Kluyveromyces lactis 21B7*
[2]: *Komagataella phaffii*
[3]: *Saccharomyces cerevisiae AH22*

Medium:
[1]: a) EnPresso Y Defined medium from Enpresso GmbH:

- 1.5 U L^{-1} Reagent A as glucose releasing enzyme were added after inoculation
- 9.0 U L^{-1} Reagent A as glucose releasing enzyme were added 8 h later
- 6.0 U L^{-1} Reagent A as glucose releasing enzyme were added after o/n cultivation

Preculture:
[1]: 10% volume of shake flask; 30°C with 250 min^{-1} and deflection of 50mm

Main cultures:
[1]: EnPresso Y Defined with start OD600 of 2 12 mL cultivation volume and different Reagent A concentrations (4,7,10 and 15 U L^{-1})
[2]: EnPresso Y Defined with 0.4% Reagent A to reach approx. 0.7 mg g^{-1} h^{-1} glucose release rate
[3]: EnPresso Y Defined at 30°C

Results

Wellenbeck et al. [1] aimed to develop a lactase production process with a non-GMO *K. lactis* strain using glucose as the sole source of carbon and energy. Single mutants, which are constitutive lactase producers isolated from chemostat cultivation, were investigated after 96-well microplate cultivations using both En-

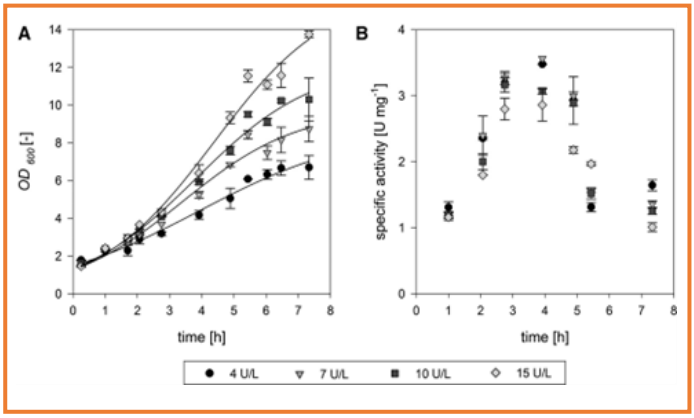

Figure 1: Parallel small-scale cultivation of K. lactis 21B7 in bioreactor 48 at four different growth rates. A) Biomass formation (OD600) and B) specific lactase activity

Presso Y Defined fed-batch medium and batch medium. It is remarkable that specific lactase activities of clones grown in EnPresso Y Defined medium showed lower scattering compared to cultivation in batch medium.

In the second phase of process development workflow, EnPresso Y Defined was employed to examine the influence of the growth rate by adding four different concentrations of the glucose releasing Reagent A (see figure 1). While biomass production increased with higher glucose release, the specific activity was not dependent on the growth rate.

Flores-Villegas et al. [2] described in their study how they received five variants of the glucose-regulated GTH1 promoter of *Komagataella phaffii.* The promoter of the glucose transporter Gth1 is tightly repressed on glucose and strongly induced in glucose-limitation and the research team did promoter engineering studies to generate promoter variants with enhanced induction strength. The effect of the promoter engineering was evaluated after small-scale screenings in 24-deep-well plates where EnPresso Y Defined was used.

Assessing microbial production strains typically occurs under uncontrolled conditions without any process monitoring. Nevertheless, dissolved oxygen (DO) stands as a pivotal factor in aerobic bioprocesses. Glauche et al. [3] present findings from their investigation using a new developed slow-responding chemo-optical sensor for DO, integrated into a 96-well plate. Figure 2 illustrates the growth curve and the DO values of S. cerevisiae cultivations for 44 h. Typical DO curves of fed-batch fermentation were recorded, the length of the oxygen limitation phase appeared to be different depending on the sensor type.

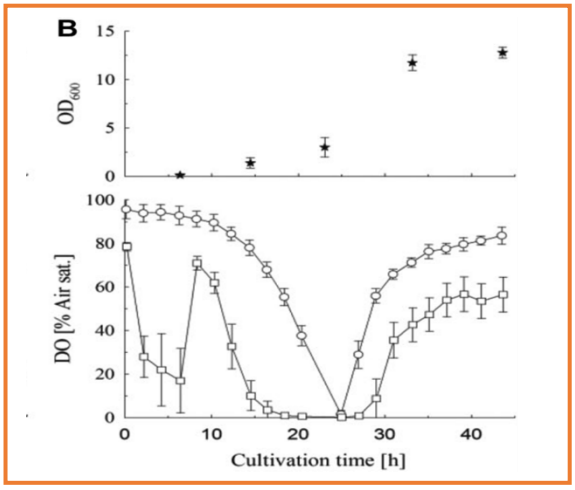

Figure 2: Application of slow- (circle) and fast- (squares) OxoPlate – Cultivation of S. cerevisiae (Glauche et al., 2015)

Conclusion

The study of Wellenbeck et al. showed that EnPresso Y Defined can be successfully applied during the early steps in process development due to applying large scale-like physiological conditions. The ultimately chosen mutant 21B7 exhibited the greatest specific lactase activity when operating in a fed-batch mode.

The application of EnPresso Y Defined was well-suited for Flores-Villegas et al. [2] to create limiting glucose (inducing) conditions for the promoter analysis. Small-scale cultivations with EnPresso Y Defined are robust and provide suitable conditions for new tools to be developed in 96-well format.

References

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[2] Flores-Villegas, M., Rebnegger, C., Kowarz, V., Prielhofer, R., Mattanovich, D. and Gasser, B. Nucleic Acids Res. 2023, 51(20), 11358-11374 https://doi.org/10.1093/nar/gkad752
[3] Glauche, F., John, G.T., Arain, S., Knepper, A., Neubauer, A., Goelling, D., Lang, C., Violet, N., Kind, R. and Neubauer, P. J Lab Autom. 2015, 20(4), 438-446 https://doi.org/10.1177/2211068215573924